

SOME OBSERVATIONS ON FUNGAL SPORES IN SOIL¹

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Work on soil mycology at Bangor started with a special interest in the Mucorales and a realization of the fact that they are nearly all wet-spored (Dobbs, 1942). From this came a curiosity about how these moulds exist and behave in the soil. Visibility is the first problem in such an investigation, and this led to trials with cellulose film to see if this would provide a suitable surface for fungal extension and growth within the soil; but the results of burying cellulose films in the soil and lifting after a few days, insufficient for any obvious exploitation of the cellulose, were disappointing as compared even with plain glass.

When protected between two glass slides however (a simple development suggested by the slide traps of La Touche, 1948) the cellulose was found to wrinkle into hollows and folds, offering a convenient observation chamber for fungal growth and sporulation. Such slide traps were used by G. D. Holmes in 1947-8, and a number of mycelia isolated from them by means of 2-mm. blocks of malt agar placed upon the hyphal tips, gave cultures of common soil moulds, mainly *Penicillium* spp., *Trichoderma* and Mucorales. More interesting were the profusion of chlamydospores and sometimes stylospores of *Mortierella* spp. formed on the cellulose film, and the frequency of animal tracks, and sometimes of the animals themselves, on the protected surfaces of the traps.

A trial of Chesters' (1940) immersion tubes also drew our attention to the importance of animal movement in the soil, as mites and small insects in particular (rather than nematodes or other worms) were found to enter the tubes via the capillary holes. Were they carrying spores? Probably, but we could not see what was happening in the tube; though in the slide traps spores could sometimes be seen germinating on the protected surfaces.

Some slide traps were lifted from a forest soil in which they had been

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buried for a period of more than 6 months. A mass of organic material, bodies and faeces of mites, nematodes and other animals, matted together with mycelium and bacteria, was found to have invaded them. The cellulose had almost disappeared. The two main impressions which we had gained up to this point were, therefore, the importance of chlamydospores in the soil, and the importance of soil animals as movers of spores and organic materials.

Realizing that the cellulose film was acting as a nutrient, we eliminated it and reduced the traps to the simplest possible devices for ensuring good visibility of whatever growth of fungi occurs on an inert, protected glass surface buried in the soil.

The all-glass slide trap

Fig. 1 shows the so-called 'gap trap' which resulted. This, simple as it is, embodies a good deal of preliminary work on the sporulation of moulds in bread and soil and in graded sands of varying pore size moistened with 2% (w/v) malt extract.

Except for the tall sporangia of *Mucor albo-ater*, sporulation of all the moulds tested occurred freely in coarse sand with cavities estimated to be of the order of 0.3 mm., but in fine sand with cavities about 0.15 mm. it was suppressed in some moulds (e.g. *Mucor hiemalis* and *M. varians*), but occurred in others (*M. albo-ater* 'Kurztrieben', *M. racemosus*, *Penicillium notatum*). *M. ramannianus* also failed to form sporangia in this fine sand, but its mycelium when recovered by flotation showed numerous chlamydospores. On the other hand, all the species tested were found to sporulate in autoclaved soil, and when pieces of bread, about $4 \times 3 \times 3$ cm., were buried in the litter layer on a woodland site, 14 days later various moulds, including *Mucor ramannianus* and *Penicillium* spp., were found to be sporing freely in the soil within a zone of about 15-cm. radius from the bread.

Tests with the slide traps buried in soil and in sterilized sand, moistened with 2% (w/v) malt extract and inoculated with a mixture of these moulds, showed that a glass-fibre spacer, 0.7 mm. thick, inserted at one end gave a suitable range of gap size for sporulation, and a suitable compromise between capillary flooding of the gap and entry of soil. In natural soil these traps gave sporulation in the gap (e.g. of *Mortierella tuberosa* and *Penicillium* spp.) only when buried within the zone of influence of some source of nutrient, e.g. 10 cm. below buried pieces of bread. Without nutrient, though there was no sporing, sterile mycelia were almost invariably found upon the protected surfaces of the glass within 10–14 days.

The method of isolation with malt agar blocks (*m*) placed on hyphal tips was repeated, but with the addition of control blocks (*c*) placed clear of the mycelium. These soon confirmed the suspicion that after 1–2 weeks in the soil the protected glass surfaces were infected all over with 'spores'. A comparison of the number of isolates from the *m* and *c* blocks led to the conclusion that at least half the mycelia on which *m* blocks had been placed were dead or unculturable, and about a third of the *m* blocks were contaminated with spores. Infection during burying and lifting was found to be negligible, but a group of 40 slide traps,

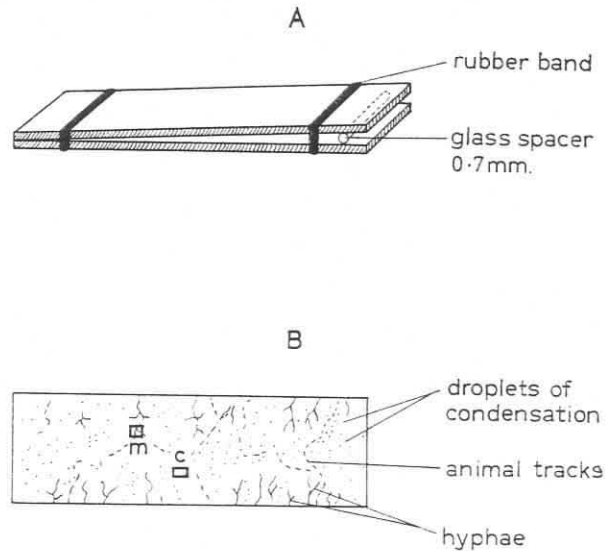


Fig. 1A. the 'gapped' Slide-Trap. B, diagram of protected side of one slide after lifting from soil. *m*, agar block placed on mycelium. *c*, agar block placed clear of mycelium.

buried together and lifted in groups of 5 at intervals up to 14 days and tested with *c* blocks, showed a progressive but erratic entry of spores. There could be little doubt about the origin of this infection, as the trails of soil animals were usually numerous and easily visible in the mist of condensation seen on the inner surfaces of the traps when lifted.

As for the isolates themselves, they were much the same set of soil moulds—species of *Penicillium*, *Cephalosporium*, *Trichoderma* and *Mucorales*, that were isolated from 'Cellophane' traps by Holmes.

All this led to a more careful examination of the origin of the mycelia in the traps. In many cases they originated on the slide surfaces, and closer inspection showed their spore origin. Some still seemed to be growing in from the edges, but when the ground edges of the slides were

examined in immersion oil, the origin of many of these could also be traced to germinating spores, and those which could not be traced to their source presented the same general appearance and staining characters as the others. Moreover, when the slide traps were merely wiped along the edges with soil or fine soil suspension, and then kept in buried Petri dishes to exclude soil animals, the appearance of the growths on them was indistinguishable from that of the ordinary buried slide trap. Altogether, there was no evidence of any invasion of the traps by mycelia growing in the soil, though the possibility could not be disproved.

This showed that our method of isolating from glass surfaces had no validity as a method of studying the growth of mycelia existing in the soil, though it might have a strictly limited value as a way of studying the moving population of water-germinable spores; and it does have a bearing on the interpretation of other methods of isolating soil fungi which involve the use of glass, or other condensing surfaces, in the soil. Why should spores germinate in such numbers on the glass when they had not done so in the soil? The only difference that we could see was that the glass, when lifted, was covered with droplets of condensed water. Could this contain some special nutrient brought in by the soil animals? This seemed unlikely, as when allowed to dry it left no visible residue on the clean glass as did soil extract, even after Seitz filtering, and also tap water. All the species commonly isolated from the traps were tested and found to germinate readily in distilled water (except some strains of *Absidia cylindrospora*). We finally realized that the soil solution must in some way inhibit germination of these spores, and this inhibition is discussed later (p. 130). Meanwhile, it seemed advisable to examine the spores in the soil as closely as possible.

'Spores' in the soil

The surfaces of agar plates were covered with 'Cellophane' discs and flooded with soil suspension from an A₂ horizon under beech. After 24 hr. incubation the discs were stripped off the agar, stained and examined under high power. There were numerous 'germlings', some of which were obviously of spore origin. Others were similar, but developed from angular-looking particles which looked like mineral particles and stained only slightly or not at all with cotton blue. Even the recognizable spores, which stained more intensely, were often irregular and indented or with mineral particles attached to them; and these graded into angular objects quite unlike the usual conception of spores. It is impossible, by the nature of the problem, to distinguish these from mineral particles by

photography or drawing, or even by sight; except that sometimes they have *curved* facets, they would not show interference effects with crossed Nicols, and would sometimes show a weak staining with gentian violet or erythrosin or a weak fluorescence when stained with acroflavine orange and examined in near U.V. light from a mercury vapour lamp.

By sedimentation and decanting a suspension of soil particles between $2\text{--}12\mu$ diameter was prepared. Apart from a few fragments of plant material, nearly all of these looked like mineral fragments; yet plating out showed that 20% of these were viable units giving rise to fungal colonies.

Sedimentation of soil 'spores'

We come now to the behaviour of these queer-looking spores during sedimentation. In Fig. 2 the graph at the top shows the number of viable

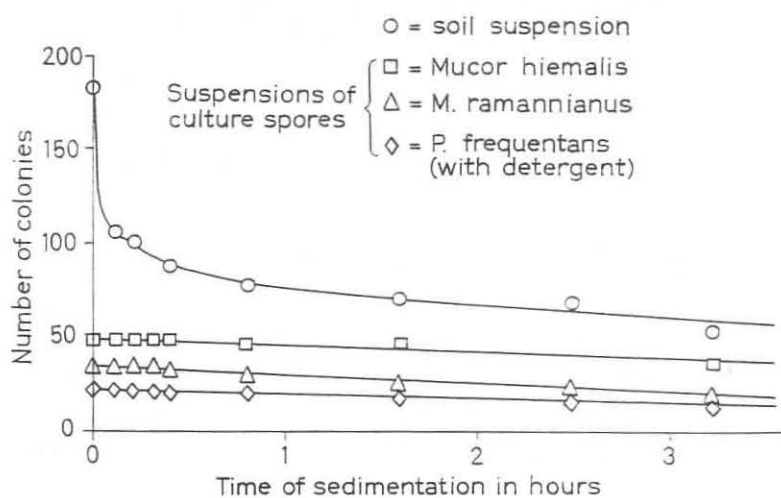


Fig. 2. Sedimentation of spores in suspension.

particles plated out from standard samples of the suspension at intervals of time during the sedimentation. Below it are similar curves for suspensions of the culture spores of two species of *Mucor* and a *Penicillium* (this last with detergent added). It will be seen that with the soil the number falls off heavily at the start, owing no doubt to the carrying down of spores with the heavier mineral particles; it then approaches the slow rate of reduction of the culture-spore suspensions. When the soil is ground up in water with a glass rod before sedimentation, the longer the grinding the less the initial fall in colonies, and the larger the proportion of spores which remain in suspension even after 2 hr.

Fig. 3 shows that the species composition of the colonies obtained by plating out did not change appreciably during the process of sedimentation. There was clearly no major sorting out of wet- and dry-spored species and, furthermore, the surface of the soil suspension, when decanted and plated out, did not show any accumulation of dry spores. In fact, it looks as if there are no xerospores in the soil, or at least in this soil (A_2 horizon under beech), despite the fact that in another experiment it was shown that the xerosporous conidia of *Penicillium nigricans* lost none of their non-wetting properties after 5 weeks of incubation, mixed with moist soil.

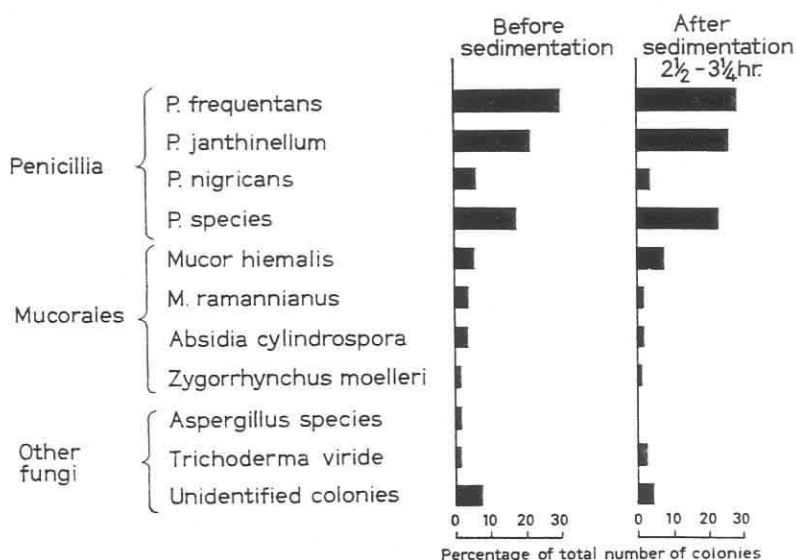


Fig. 3. Frequency of species in soil suspension before and after sedimentation.

To test this further, a simple variant was tried of Burges' experiment (1950) with the movement of spores through a sand column. A Buchner funnel packed with sand showed that wet spores (*M. hiemalis*) passed through it, but dry spores (*Penicillium frequentans*) were retained, forming a surface scum which reappeared with a rising water-level. When a soil suspension was passed through, no such differentiation occurred. *Penicillia* were numerous in the effluent, as tested by plating out, and if the colonies came from conidia, they must have lost their unwettable surface properties.

It has been shown (Burges and Hepple, 1954) that culture spores will not percolate through fine soil, as distinct from sand, and Hinson further showed that, with soil in the funnel, even under gentle suction

95% of the viable spores in a soil suspension are retained; so it did seem reasonable to suppose that, so far as the movement of water is concerned, the layers of the soil might retain most of the spores which fall on them or are formed in them, until they die or germinate. The next step, therefore, was to investigate the distribution of spores in undisturbed soil to see what record might be retained there of sporulation and survival of spores.

Distribution of spores in clods of soil

Clods of silty loam were carefully removed, with the top marked, from the A₂ horizon under beech which had been studied. In the labora-

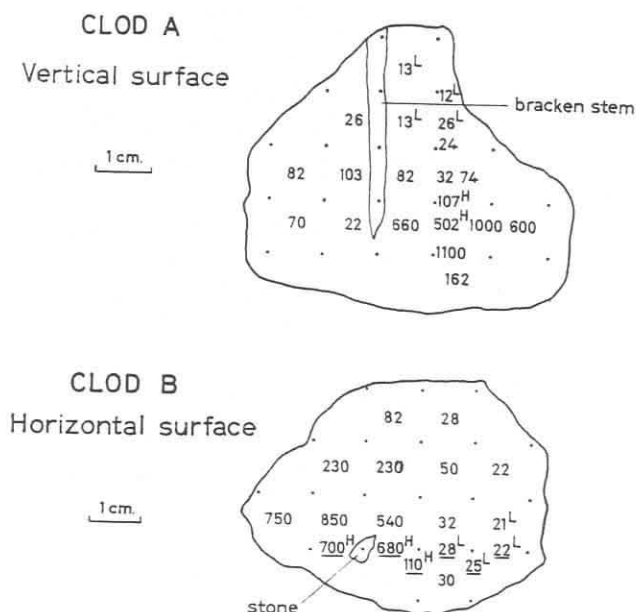


Fig. 4. Distribution of 'spore' population over surfaces of two adjacent clods of soil.

Diagrams are scale drawings of surfaces examined. Dots represent markers; figures represent colonies per mg. of soil referred to points of origin of samples; figures underlined were from samples taken 14 days later; plates from samples marked H or L were used to enumerate the species in areas of high and low 'spore' density respectively.

tory these were broken across to expose fresh surfaces, and these surfaces were marked out in 1-cm. squares with glass fibres as markers. From the centre of each square a minute sample was removed on the flattened end of a needle and weighed on a glass-fibre balance. These little crumbs varied in weight from 4 to 29 mg. Each was carefully ground up for 5 min. in a measured amount (1 ml. per mg.) of water, sedimented for 5 min., and a 0.5 ml. sample was plated out. In some cases further

standard dilutions were necessary to get a count. Colonies were counted after 14 days, and a wide range of spore density (no. per mg. soil) was disclosed. These were recorded in the appropriate squares on a scale diagram of the clod surface (as shown in Fig. 4). In some cases a 'transect' of closer samples was taken across the clod surface to confirm the variation of high and low density. This intricate technique took a good deal of practice to make perfect, and was repeated on a number of clods. Fig. 4 shows two typical examples of the results from *adjacent* clods, broken vertically and horizontally, respectively. The variation in spore density within each clod is very marked, even within 1 or 2 cm.

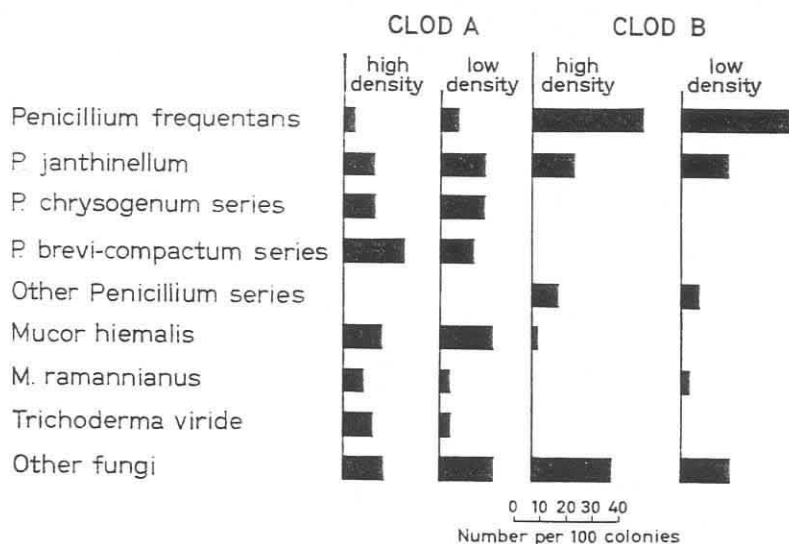


Fig. 5. Frequency of species in zones of high and low spore density.

Fig. 5 shows roughly the species composition on certain plates taken from zones of high and low density in these two neighbouring clods of soil. It seems that none of these few-milligram samples came from a point of sporulation of one species. The composition of the spore population was mixed, but substantially the same at all points—of high and low spore density—within the clods; but a few inches away in the other clod the composition had changed somewhat.

As a further test, broken clod surfaces were inoculated at marked points with spores of several moulds, some dry, some in water, some in malt extract. The two halves were carefully fitted together and incubated for 2–4 weeks; then separated again and examined at the marked points with a Leitz Ultropak. Where no nutrient had been added spores could be seen but no germination, but at the 'malt' points germlings were to

be seen after 24 hr., and extensive mycelia after 2 weeks, with sporangia of *Mucor ramannianus* where that species had been inoculated.

The soil picture

From these observations added to those of many other workers, we are beginning to get the barest outline of a picture of the biology of these common ephemeral moulds in the soil. It seems reasonable to assume that at least in moist, well-aerated soil and at suitable temperatures, they are able to spore in the soil wherever there is sufficient nutrient and cavities of the order of 200μ . Some of them also form numerous chlamydospores, on the survival of which in soil Caldwell (1958) has recently thrown some light. But these local accumulations of spores must be very ephemeral, for they seem to be subjected to a continual local churning and mixing which gives every soil crumb an almost uniform species composition within a clod of a few centimetres, although it varies somewhat when we explore on a larger scale.

This mixing process would apply also to spores washed or carried down from the surface layer, although, except in freely draining soils, the washing down of spores in rain-water would mainly occur along vertical fissures in the soil. As for the agency which performs this mixing and churning, the continual invasion of the slide traps by soil animals and by spores leaves no doubt about it. The important paper of Hartmann (1944) revealed the enormous part played in forest soils by the fauna and their excreta, which indeed can make up a large proportion of the soil organic matter; but the extent to which the soil-spore pattern varies in mull and mor soils, dominated by different animals, and in soils which restrict animal movement, remains at present uninvestigated.

To what extent these points of high but mixed spore density and these queer angular shapes which spores take up in the soil, and their lack of non-wetting surfaces, are associated with passage through the animal's gut; and to what extent they are merely adaptations to soil surfaces and cavities, remains to be discovered, as also does the proportion of chlamydospores to conidia and sporangiospores. In addition to the 20% of soil particles in suspension between $2-12\mu$ in size which germinate quickly to give mould colonies, how many of the others are not mineral particles but chlamydospores, basidiospores, or other spores with special germination requirements, not to mention cysts of algae, protozoa, or other organisms? We are still very far from getting a clear picture, but the shadowy outline is beginning to be very interesting.

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